46. Wigham CG, Turner HC, Swan J, Hodson SA. Modulation of corneal endothelial hydration control mechanisms by Rolipram. *Pflugers Arch-Eur J Physiol.* 2000;440:866-870.
47. Mimura T, Yamagami S, Yokoo S, et al. Cultured human corneal endothelial cell transplantation with a collagen sheet in a rabbit model. *Invest Ophthalmol Vis Sci.* 2004;45:2992-2997.
48. Chibalin AV, Ogimoto G, Pedemonte CH, et al. Dopamine-induced endocytosis of Na^+,K^+-ATPase is initiated by phosphorylation of Ser18 in the rat α_1 subunit and is responsible for the decreased activity in epithelial cells. *J Biol Chem.* 1999;274:1920-1927.
49. Yudowski GA, Efendiev R, Pedemonte CH, et al. Phosphoinositide-3 kinase binds to a proline-rich motif in the Na^+,K^+-ATPase α_1 subunit and regulates its trafficking. *Proc Natl Acad Sci U S A.* 2000;97:6556-6561.
50. Feiz V. Corneal edema. In: Krachmer JH, Mannis MJ, Holland EJ, eds. *Cornea.* 2nd ed. London: Elsevier Mosby; 2005:359-363.
51. Jorgensen PL, Hakansson KO, Karlish SJ. Structure and mechanism of Na,K-ATPase: functional sites and their interactions. *Annu Rev Physiol.* 2003;65:817-849.
52. Lingrel J, Moseley A, Dostanic I, et al. Functional roles of the alpha isoforms of the Na,K-ATPase. *Ann N Y Acad Sci.* 2003;986:354-359.
53. Huang B, Blanco G, Mercer RW, et al. Human corneal endothelial cell expression of Na^+,K^+-adenosine triphosphatase isoforms. *Arch Ophthalmol.* 2003;121:840-845.
54. Feschenko MS, Sweadner KJ. Conformation-dependent phosphorylation of Na,K-ATPase by protein kinase A and protein kinase C. *J Biol Chem.* 1994;269:30436-30444.
55. Feschenko MS, Sweadner KJ. Structual basis for species-specific differences in the phosphorylation of Na,K-ATPase by protein kinase C. *J Biol Chem.* 1995;270:14072-14077.
56. Feschenko MS, Stevenson E, Sweadner KJ. Interaction of protein kinase C and cAMP-dependent pathways in the phosphorylation of the Na,K-ATPase. *J Biol Chem.* 2000;275:34693-34700.
57. Hatou S, Yamada M, Mochizuki H, et al. Role of protein kinase C in regulation of Na^+- and K^+-dependent ATPase activity and pump function in corneal endothelial cells. *Jpn J Ophthalmol.* 2009;53:235-242.
58. Ragolia L, Cherpalis B, Srinivasan M, et al. Role of serine/threonine protein phosphatases in insulin regulation of Na^+/K^+-ATPase activity in cultured rat skeletal muscle cells. *J Biol Chem.* 1997;272:23653-23658.
59. Srinivasan M, Begum N. Stimulation of protein phosphatase-1 activity by phorbol esters: evaluation of the regulatory role of protein kinase C in insulin action. *J Biol Chem.* 1994;269:16662-16667.
60. Srinivasan M, Begum N. Regulation of protein phosphatase 1 and 2A activities by insulin during myogenesis in rat skeletal muscle cells in culture. *J Biol Chem.* 1994;269:12514-12520.
61. Civan MM, Peterson-Yantorno K, O'Brien TG. Insulin and phorbol ester stimulate conductive Na^+ transport through a common pathway. *Proc Natl Acad Sci U S A.* 1988;85:963-967.
62. Efendiev R, Pedemonte CH. Contrary to rat-type, human-type Na,K-ATPase is phosphorylated at the same amino acid by hormones that produce opposite effects on enzyme activity. *J Am Soc Nephrol.* 2006;17:31-38.
63. Feraille E, Carranza ML, Gonin S, et al. Insulin-induced stimulation of Na+, K+-ATPase activity in kidney proximal tubule cells depends on phosphorylation of the α-subunit at Tyr-10. *Mol Biol Cell.* 1999;10:2847-2859.
64. Al-Khalili L, Kotova O, Tsuchida H, et al. ERK1/2 mediates insulin stimulation of Na,K-ATPase by phosphorylation of the α-subunit in human skeletal muscle cells. *J Biol Chem.* 2004;279:25211-25218.
65. Cho JY, Park J. Contribution of natural inhibitors to the understanding of the PI3K/PDK1/PKB pathway in the insulin-mediated intracellular signaling cascade. *Int J Mol Sci.* 2008;9:2217-2230.
66. Liu L, Zhao X, Pierre V, et al. Association of PI3K-Akt signaling pathway with digitalis-induced hypertrophy of cardiac myocytes. *Am J Physiol Cell Physiol.* 2007;293:1489-1497.
67. Tian J, Li X, Liang M, et al. Changes in sodium pump expression dictate the effects of ouabain on cell growth. *J Biol Chem.* 2009;29;284:14921-14929.

Comparison of Anterior and Posterior Corneal Surface Irregularity in Descemet Stripping Automated Endothelial Keratoplasty and Penetrating Keratoplasty

Takefumi Yamaguchi, MD,† Kazuno Negishi, MD,* Kazuko Yamaguchi, MD,* Murat Dogru, MD,*‡ Yuichi Uchino, MD,* Shigeto Shimmura, MD,* and Kazuo Tsubota, MD**

Purpose: To evaluate the irregularity of the anterior and posterior cornea after Descemet stripping automated endothelial keratoplasty (DSAEK) and penetrating keratoplasty (PK).

Methods: This clinical study comprised 39 eyes: 13 consecutive eyes after DSAEK, 13 consecutive eyes after PK, and 13 age-matched normal eyes. Corneal elevation data were acquired using a rotating Scheimpflug camera 1 and 3 months after DSAEK and PK. Anterior and posterior corneal elevation data were decomposed into a set of Zernike polynomials up to eighth order. Total higher-order root mean square (RMS) and RMS from third to eighth order were calculated. The astigmatism and irregularity of the anterior and posterior surfaces were compared between DSAEK and PK.

Results: The regular astigmatism and tilt components of the anterior surface were significantly lower after DSAEK than after PK at 1 and 3 months ($P < 0.001$), whereas there was no difference in astigmatism of the posterior surface between the groups ($P = 0.07$, 0.22). The higher-order RMS and RMSs of third- to eighth-order components of the anterior surface were significantly larger after PK than those after DSAEK at 1 and 3 months ($P < 0.01$), whereas there were no significant differences between DSAEK and PK in higher-order aberration RMS and RMSs of third- to eighth-order components of the posterior surface.

Conclusions: Postoperative corneal irregularity of the anterior surface was greater after PK than after DSAEK, whereas there was no significant difference in posterior surface irregularity. DSAEK is superior to PK in terms of the higher-order irregularity of the anterior surface.

Key Words: DSAEK, penetrating keratoplasty, astigmatism, irregular astigmatism, anterior corneal surface, posterior corneal surface

(*Cornea* 2010;29:1086–1090)

Descemet stripping automated endothelial keratoplasty (DSAEK) for the treatment of dysfunctional endothelium has several advantages over standard penetrating keratoplasty (PK).[1–5] By removing only Descemet membrane and endothelium and retaining the healthy portions of the patients' cornea, DSAEK offers rapid visual recovery and preservation of the corneal biomechanical properties and integrity. Whereas DSAEK is an excellent selective transplantation that has improved the postoperative visual prognosis in patients with bullous keratopathy, it is reported that the majority gain 20/40 vision, but a few gain 20/20 vision. Interface scatter and age are possible factors that affect postoperative visual acuity.[6] In our previous report, the anterior surface irregularity after DSAEK, not the irregularity of the posterior surface, has an important effect on visual acuity,[7] which indicates that, in addition to the corneal transparency, regularity of the anterior surface is an important factor for visual acuity after DSAEK. Postoperative irregularity of the anterior and posterior surfaces must be one of the important factors in selection of DSAEK or PK for the treatment of bullous keratopathy. However, there has been no reported comparison of irregularity between DSAEK and PK. Therefore, we compared the irregularity of anterior and posterior surfaces after DSAEK and PK.

MATERIALS AND METHODS

This study included 39 eyes, 13 age-matched normal eyes without apparent corneal disease and 26 eyes of consecutive patients who underwent DSAEK (13 eyes) and PK (13 eyes) at Keio University Hospital. This retrospective study was approved by the Institutional Review Board of Keio University Hospital. Written informed consent was obtained from all patients. This study adhered to the tenets of the Declaration of Helsinki.

The pre- and postoperative clinical data are shown in Table 1. The average age was 73.7 ± 7.8 years (from 59 to 85 years old; 7 women and 6 men) in the DSAEK group and 62.5 ± 33.5 years (from 31 to 86 years old; 4 women and 9 men) in the PK group. There were no significant differences in age between the groups. The average age of normal patients

Received for publication October 9, 2009; revision received December 4, 2009; accepted December 9, 2009.

From the *Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan; †Department of Ophthalmology, Tokyo Dental College, Chiba, Japan; and ‡J&J Ocular Surface and Visual Optics Department, Keio University School of Medicine, Tokyo, Japan.

The authors have no proprietary interest in any of the devices mentioned in the article.

Reprints: Takefumi Yamaguchi, Department of Ophthalmology, Keio University School of Medicine, Shinanomachi 35, Shinjuku-ku, Tokyo 1608513, Japan (e-mail: yama19770614@hotmail.com).

TABLE 1. Patients' Data

	DSAEK Group (n = 13)	PK Group (n = 13)	*P*
Age (yrs)	73.7 ± 7.8	62.5 ± 33.5	0.350
Sex			
Female	7	4	
Male	5	9	
Preoperative			
BCVA (logMAR)	1.15 ± 0.49	1.03 ± 0.58	0.289
Spherical equivalent (D)	−1.7 ± 2.1	−2.7 ± 3.1	0.238
Astigmatism (D)	−2.5 ± 1.1	−3.5 ± 2.7	0.072
Postoperative (1 mo)			
BCVA (logMAR)	0.52 ± 0.28	0.58 ± 0.52	0.362
Spherical equivalent (D)	−0.1 ± 1.7	−1.5 ± 2.5	0.052
Astigmatism (D)	−2.3 ± 1.3	−5.5 ± 2.6	<0.001
Corneal thickness (μm)	601 ± 85	562 ± 58	0.10
Postoperative (3 mo)			
BCVA (logMAR)	0.42 ± 0.33	0.49 ± 0.43	0.34
Spherical equivalent (D)	−0.43 ± 2.1	−1.6 ± 2.5	0.11
Astigmatism (D)	−1.9 ± 1.1	−3.7 ± 2.1	<0.001
Corneal thickness (μm)	596 ± 96	529 ± 42	0.019

BCVA, best-corrected visual acuity; D, diopter.

was 65.8 ± 14.4 years (from 35 to 79 years old; 1 man and 12 women). The preoperative logarithm of the minimum angle of resolution (logMAR) was 1.15 ± 0.49 in the DSAEK group and 1.03 ± 0.58 in the PK group.

The causes of bullous keratopathy in the DSAEK group were Fuchs endothelial dystrophy (7 eyes), pseudophakic bullous keratopathy (3 eyes), and bullous keratopathy caused by laser iridotomy (3 eyes). The indications for the PK group included bullous keratopathy (8 eyes), keratoconus (3 eyes), and corneal stromal opacity (2 eyes).

We performed DSAEK according to our standard technique, as previously published. Briefly, after sub-Tenon anesthesia with injection of 2% lidocaine, a 5.0-mm corneoscleral incision was made at the 12-o'clock position. Two paracenteses were made at the 7- and 10-o'clock positions. An anterior chamber maintenance cannula was inserted through the 7-o'clock paracentesis, and Descemet stripping was performed for a diameter of 8.0 mm with a reverse-bent Sinskey hook (Asico, Westmont, IL) corresponding to the 8.0-mm epithelial trephine marker. The recipient's endothelium and Descemet membrane were carefully removed by forceps. Donor precut tissue was obtained from SightLife Eye Bank of Seattle. Precut donors were trephinated at a size of 8.0 mm, and the endothelial surface of the donor lenticle was coated with a small amount of viscoelastic material (Viscoat; Alcon, Fort Worth). Donor tissue was gently folded using forceps and inserted into the anterior chamber. Air was carefully injected into the anterior chamber to unfold the graft. The fluid between the recipient's stroma and the graft was drained from small incisions in the midperipheral recipient cornea. Ten minutes after the air injection, most of the air was replaced with balanced salt solution (Alcon). At the end of the surgery, subconjunctival tobramycin and betamethasone were administered.

PK was performed under retrobulbar anesthesia. The donor button was cut with a Barron punch trephine (diameter, 7.75 mm in all eyes). The recipient bed was 7.5 mm in all cases. A Hessburg–Barron suction trephine was used to cut a partial-depth circular incision in the cornea, centered at the geometric center of the cornea. Excision of the recipient corneal button was completed with curved corneal scissors. The graft was sutured in place with a single-running 10-0 nylon suture with 24 bites. At the end of the surgery, subconjunctival tobramycine and betamethasone were administered.

The corneal topography of the anterior and posterior surfaces was measured using a Pentacam (Oculus, Inc, Wetzlar, Germany). The Pentacam uses a rotating Scheimpflug camera to analyze the anterior segment anatomy. In this study, the Pentacam's 50-picture 3-dimensional scan measurement mode was used. The Pentacam automatically captured images once correct alignment in the x, y, and z directions was attained. After image capture, the instrument analyzed the 50 Scheimpflug images of the anterior eye and allowed export of corneal elevation topographic data (anterior and posterior corneal surfaces). The Pentacam analysis provided data from an area of up to 10 mm in diameter, which included the graft size of 8 mm in this study. Data from the central 4-mm circle were analyzed. No subjects wore contact lenses before and after surgery. The Scheimpflug images were taken 1 and 3 months after DSAEK and PK.

The topographic data of the anterior and posterior surfaces taken by the Pentacam were decomposed into a set of Zernike polynomials. The Zernike coefficients were determined up to eighth order for a 4-mm diameter. The root mean square (RMS) of $Z(2, -2)$ and $Z(2, 2)$ was calculated to represent the lower-order line symmetric component and defined as astigmatism in this study. The RMS of the total higher-order coefficients from third to eighth order was calculated to represent the irregular component of astigmatism and defined as higher-order RMS. We evaluated the postoperative $Z(2, 0)$, astigmatism, RMSs of third to eighth order, and higher-order RMS. The central corneal thickness was measured by the Pentacam. The logarithm of visual acuity was calculated and analyzed statistically. A P value less than 0.05 was considered statistically significant. Comparison of logMAR, corneal thickness, and RMSs of topographic data between DSAEK and PK was performed using Mann–Whitney U test. All statistical analyses were performed with (SAS Institute, Inc, Cary, NC) computer software.

RESULTS

The clinical data are shown in Table 1. The postoperative astigmatism was significantly lower in the DSAEK group than that in the PK group ($P < 0.001$). The postoperative central corneal thickness was significantly larger in the DSAEK group than that in the PK group at 3 months ($P = 0.019$).

Table 2 shows the average of lower-order Zernike coefficients of the anterior surface after DSAEK and PK. In the DSAEK group, although there was a significant difference in astigmatism compared with the normal eye group at 1 month, there were no significant differences in astigmatism and tilt at 3 months. The astigmatism and tilt of the PK group were

TABLE 2. The Average of Lower Order of Zernike Coefficients of Anterior Corneal Surface After DSAEK and PK

	Postoperative (1 Mo)	Postoperative (3 Mo)
DSAEK		
Spherical $Z(2,0)$	150.7 ± 8.3	151.8 ± 8.7
Astigmatism	6.3 ± 4.0†	5.5 ± 4.0
Tilt $Z(11)$	7.1 ± 7.0	5.3 ± 3.3
PK		
Spherical $Z(2,0)$	157.5 ± 19.3	154.7 ± 14.0
Astigmatism	21.7 ± 9.6¶†	16.2 ± 10.1¶†
Tilt $Z(11)$	37.6 ± 19.7¶†	29.3 ± 13.3¶†

Normal eyes (n = 13): spherical $Z(2, 0)$ = 153.5 ± 7.1, astigmatism = 3.3 ± 2.2 D, and tilt $Z(11)$ = 4.8 ± 4.4.

¶$P < 0.05$ compared with the DSAEK group, †$P < 0.05$ compared with the normal eye group.

Mean ± SD (μm).

significantly larger than those of the DSAEK and normal eye groups at 1 and 3 months ($P < 0.05$).

Table 3 shows the average of the lower-order Zernike coefficients of the posterior surface after DSAEK and PK. The spherical component, astigmatism, and tilt of the DSAEK and PK groups were significantly larger than those of the normal eye group at 1 month. There were no differences in spherical aberration, astigmatism, and tilt between the DSAEK and normal eye groups at 3 months. The lower-order Zernike coefficients 3 months after PK were significantly larger than those of the normal eye group.

Table 4 shows the average of the higher-order Zernike coefficients of the anterior surface after DSAEK and PK. The higher-order Zernike coefficients after PK were significantly larger than those after DSAEK and of the normal eye group. There were no significant differences in higher-order Zernike coefficients between the DSAEK and normal eye groups, except for the sixth-order RMS at 1 and 3 months and the eighth-order RMS at 1 month after DSAEK.

Table 5 shows the average of the higher-order Zernike coefficients of the posterior surface after DSAEK and PK. The higher-order Zernike coefficients after DSAEK and PK were significantly larger than those of the normal eye group.

TABLE 3. The Average of Lower Order of Zernike Coefficients of Posterior Corneal Surface After DSAEK and PK

	Postoperative (1 Mo)	Postoperative (3 Mo)
DSAEK		
Spherical $Z(2, 0)$	209.5 ± 20.0†	181.7 ± 31.4
Astigmatism	19.6 ± 5.7†	14.1 ± 7.5
Tilt $Z(11)$	71.7 ± 40.6†	43.8 ± 41.4
PK		
Spherical $Z(2, 0)$	209.3 ± 15.2†	204.0 ± 15.2¶†
Astigmatism	26.4 ± 14.4†	18.9 ± 11.7†
Tilt $Z(11)$	42.9 ± 14.0¶†	45.1 ± 19.8†

Normal eyes (n = 13): spherical $Z(2, 0)$ = 190.9 ± 13.7, astigmatism = 9.5 ± 6.5 D, and tilt $Z(11)$ = 31.3 ± 8.4.

¶$P < 0.05$ compared with the DSAEK group, †$P < 0.05$ compared with the normal eye group.

Mean ± SD (μm).

TABLE 4. The Average of Higher-Order RMS of Zernike Coefficients of Anterior Corneal Surface After DSAEK and PK

	Postoperative (1 Mo)	Postoperative (3 Mo)
DSAEK		
HO-RMS	2.92 ± 2.10	2.54 ± 1.29
S3	2.33 ± 2.06	2.05 ± 1.18
S4	1.50 ± 0.80	1.36 ± 0.64
S5	0.49 ± 0.27	0.43 ± 0.18
S6	0.21 ± 0.14†	0.19 ± 0.10†
S7	0.05 ± 0.06	0.02 ± 0.04
S8	0.01 ± 0.02†	0.00 ± 0.01
PK		
HO-RMS	11.9 ± 5.91¶†	9.86 ± 4.50¶†
S3	10.1 ± 6.03¶†	8.49 ± 4.92¶†
S4	5.28 ± 2.1¶†	4.04 ± 1.08¶†
S5	1.98 ± 1.46¶†	1.71 ± 0.78¶†
S6	0.70 ± 0.65¶†	0.57 ± 0.37¶†
S7	0.26 ± 0.46¶†	0.13 ± 0.13¶†
S8	0.05 ± 0.07¶†	0.04 ± 0.05¶†

Normal eyes (n = 13): HO-RMS = 2.11 ± 0.78, S3 = 1.58 ± 0.67, S4 = 1.23 ± 0.49, S5 = 0.41 ± 0.39, S6 = 0.11 ± 0.07, S7 = 0.02 ± 0.04, and S8 = 0.00 ± 0.00.

¶$P < 0.05$ compared with the DSAEK group, †$P < 0.05$ compared with the normal eye group.

HO-RMS, higher-order RMS.

Mean ± SD (μm).

However, there were no differences in RMSs of the higher-order Zernike coefficients between the DSAEK and PK groups at 1 and 3 months.

DISCUSSION

The important advantage of DSAEK is that it causes little to no change in corneal refractive cylinder compared with PK.[1,8,9] However, the differences in the irregularity of the corneal anterior and posterior surfaces after DSAEK and PK have remained unknown. We reported that the irregularity of the anterior and posterior surfaces decreases after DSAEK, and it is the irregularity of the anterior surface that has an effect on postoperative visual acuity.[7] In this study, we evaluated the difference in the postoperative irregularity of the anterior and posterior surfaces after DSAEK and PK.

Comparing the outcomes of DSAEK and PK, the advantages of DSAEK are rapid visual recovery, lower astigmatism, stability in the postoperative refraction, lower rejection rate, and integrity of the wound structure.[5,9] Hjortdal et al evaluated the efficacy of DSAEK for the treatment of Fuchs endothelial dystrophy and reported that, compared with PK, visual recovery is faster and astigmatism is not induced after DSAEK; however, primary graft failure may be common. In their report, the best-corrected visual acuity at 12 months after DSAEK was significantly better than that after PK.[5] Bahar et al[9] reported that DSAEK enabled rapid and better uncorrected visual acuity and best-corrected visual acuity when compared with PK with significantly lower astigmatism. As for the comparison in regular astigmatism and tilt of the anterior surface between DSAEK and PK, the astigmatism was

TABLE 5. The Average of Higher-Order RMS of Zernike Coefficients of Posterior Corneal Surface After DSAEK and PK

	Postoperative (1 Mo)	Postoperative (3 Mo)
DSAEK		
HO-RMS	17.6 ± 9.2†	11.0 ± 8.56†
S3	14.0 ± 8.68†	8.67 ± 7.67†
S4	9.44 ± 4.20†	5.82 ± 3.96†
S5	3.08 ± 2.03†	1.93 ± 1.69†
S6	1.43 ± 1.17†	0.97 ± 1.09†
S7	0.26 ± 0.19†	0.16 ± 0.15†
S8	0.07 ± 0.06†	0.04 ± 0.09
PK		
HO-RMS	12.0 ± 5.82†	10.7 ± 4.64†
S3	9.79 ± 3.59†	8.24 ± 5.24†
S4	7.13 ± 3.61†	5.41 ± 1.80†
S5	2.92 ± 1.29†	2.31 ± 1.26†
S6	1.27 ± 1.03†	0.90 ± 0.48†
S7	0.27 ± 0.23†	0.25 ± 0.16†
S8	0.10 ± 0.11†	0.10 ± 0.13†

Normal eyes (n = 13): HO-RMS = 3.85 ± 0.95, S3 = 3.13 ± 1.20, S4 = 1.95 ± 0.37, S5 = 0.68 ± 0.32, S6 = 0.23 ± 0.09, S7 = 0.06 ± 0.02, and S8 = 0.02 ± 0.02.

¶$P < 0.05$ compared with the DSAEK group, †$P < 0.05$ compared with the normal eye group.

HO-RMS, higher-order RMS.

Mean ± SD (μm).

significantly lower in DSAEK than in PK, as reported in previous studies,[3,9–11] which seems reasonable because the anterior surface of the cornea is retained in DSAEK. The tilt components of the anterior and posterior surfaces after PK or DSAEK have not been reported previously. We demonstrated that the tilt of the anterior surface in DSAEK was significantly lower than that in PK, although the tilt components of the posterior surface in DSAEK and PK were similar and significantly larger than that in normal eyes. The causes of the tilt component may be eccentric graft preparation, intraoperative decentration of the recipient trephination, and the patients' eye fixation during the examination. It remains unknown whether the corneal tilt component might affect postoperative vision. Manual preparation of the donor and recipient corneas may cause tilt; therefore, keratoplasty using new technology such as femtosecond lasers may make it possible to prepare a precise corneal graft and recipient's corneal cut,[12–18] which may decrease the tilt and other irregular components. Further evaluation of the causes of the postoperative tilt component and its effect on visual acuity is necessary in the future.

In the current study, we demonstrated that the higher-order irregularity of the anterior surface after DSAEK was significantly lower than that after PK, although there was no significant difference in posterior surface between the groups. This is the first report that evaluated the postoperative corneal irregularity of both anterior and posterior surfaces after DSAEK and PK. Terry and Ousley[19] evaluated the topography using TMS and Orbscan after deep lamellar endothelial keratoplasty (DLEK) and reported that DLEK preserves normal corneal topography of the anterior surface and minimizes postoperative astigmatism. McLaren et al[20] evaluated higher-order aberration (HOA) after DLEK and PK and reported that HOAs from the anterior surface were higher after PK than after DLEK. Hindman et al[21] evaluated the light scatter and wavefront aberration after DLEK in 4 patients and reported that the wavefront aberration was lower after DLEK than after PK but that the light scatter because of corneal haze was higher after DLEK than after PK. In our previous report, we demonstrated that the higher-order irregularity of the anterior surface is negatively correlated with postoperative visual acuity.[7] This indicates that, although there are individual differences in anterior surface higher-order irregularity for unknown reasons after DSAEK, its average values were smaller compared with those after PK. In this current study, there was no difference in postoperative logMAR between DSAEK and PK, although the irregularity of the anterior surface was significantly smaller in DSAEK than in PK. The causes of poor visual outcome after DSAEK would be multifactorial: age, light scatter, and irregular astigmatism of the anterior surface as have been reported in the past.[6,7] More comprehensive study on vision-limiting factors measuring corneal opacity, duration of bullous keratopathy, light scatter, and irregular astigmatism after DSAEK and PK should be substantiated in the future.

Evaluation of the corneal posterior surface has been reported by several authors.[2,23] However, the exact level of regular and irregular astigmatism that affects the visual function is unknown. Dubbelman et al[22] reported that the astigmatism of the posterior surface reduces than that of the anterior surface by 31%, which means that the posterior surface has a considerable impact on the total astigmatism. Dubbelman et al[24] calculated the comatic aberration of the whole cornea from a Scheimpflug imaging reconstruction of a normal cornea and concluded that it is sufficient to take only the anterior corneal surface into account because the comatic aberration of the posterior surface is small compared with that of the anterior surface. Sicam et al[23] reported that the spherical aberration of the posterior surface changes with age. Oshika et al[25] reported that the posterior to anterior ratios of the regular and irregular astigmatism are 35.0% ± 41.3% and 39.9% ± 39.9%, respectively, in normal eyes. However, the impact of the posterior corneal surface on vision has not been explained sufficiently, although 2 previous case reports suggested the possible influence of posterior corneal curvature on the visual function.[26,27] Although the irregularity of the posterior surface increased up to the post-PK level after DSAEK in this study, the irregularity of the posterior surface might not affect visual acuity because the small refractive index change between the cornea and aqueous humor is relatively small compared with the change between air and the cornea.

The limit of the current study is that short-term results of postoperative 3 months were examined. Longitudinal studies are needed to follow the long-term alterations in the irregularities of the anterior and posterior surfaces of the cornea after DSAEK and PK. The accuracy of the Pentacam used for the evaluation of the corneal surfaces after DSAEK and PK has not been fully validated. Although the Pentacam, which is a relatively new instrument that images the anterior and posterior corneal surfaces by employing a rotating Scheimpflug camera,

may have some limitations, measurements of corneal thickness and posterior elevation with the Pentacam have been reported to be highly reproducible and repeatable.[28–30] Another limitation of this study is the application of Zernike polynomial functions for the analysis of topographic data of corneal surfaces, not HOA. The validity of Zernike polynomial functions to represent the corneal surface might be a subject that requires more study. Smolek and Klyce[31] evaluated the corneal topography of normal eyes and eyes after PK, radial keratotomy, and keratoconus and reported that the fourth-order Zernike polynomial seemed reliable for modeling the normal cornea and that if more Zernike terms were added, the accuracy of the fit to the abnormal cornea will be improved to the degree of the minimum errors found in normal corneas. We evaluated the corneal surfaces using Zernike polynomials up to eighth order. The eighth-order values after DSAEK and PK were very small or nearly zero, which may indicate the validity of the fit to the abnormal surfaces by eighth-order Zernike polynomials and may sufficiently reflect the true surface values. More comprehensive studies should be performed in future.

In conclusion, the irregularity of the anterior surface was significantly smaller after DSAEK compared with that after PK, whereas there was no significant difference in the posterior surface irregularity. DSAEK is superior to PK in terms of the higher-order irregularity of the anterior surface.

REFERENCES

1. Price MO, Price FW Jr. Descemet's stripping with endothelial keratoplasty: comparative outcomes with microkeratome-dissected and manually dissected donor tissue. *Ophthalmology.* 2006;113:1936–1942.
2. Price FW Jr, Price MO. Descemet's stripping with endothelial keratoplasty in 50 eyes: a refractive neutral corneal transplant. *J Refract Surg.* 2005;21: 339–345.
3. Price MO, Price FW. Descemet's stripping endothelial keratoplasty. *Curr Opin Ophthalmol.* 2007;18:290–294.
4. Koenig SB, Covert DJ. Early results of small-incision Descemet's stripping and automated endothelial keratoplasty. *Ophthalmology.* 2007; 114:221–226.
5. Hjortdal J, Ehlers N. Descemet's stripping automated endothelial keratoplasty and penetrating keratoplasty for Fuchs' endothelial dystrophy. *Acta Ophthalmol.* 2009;87:310–314.
6. Patel SV, Baratz KH, Hodge DO, et al. The effect of corneal light scatter on vision after Descemet stripping with endothelial keratoplasty. *Arch Ophthalmol.* 2009;127:153–160.
7. Yamaguchi T, Negishi K, Yamaguchi K, et al. Effect of anterior and posterior corneal surface irregularity on vision after Descemet-stripping endothelial keratoplasty. *J Cataract Refract Surg.* 2009;35:688–694.
8. Price FW Jr, Price MO. Descemet's stripping with endothelial keratoplasty in 200 eyes: early challenges and techniques to enhance donor adherence. *J Cataract Refract Surg.* 2006;32:411–418.
9. Bahar I, Kaiserman I, McAllum P, et al. Comparison of posterior lamellar keratoplasty techniques to penetrating keratoplasty. *Ophthalmology.* 2008; 115:1525–1533.
10. Terry MA, Shamie N, Chen ES, et al. Precut tissue for Descemet's stripping automated endothelial keratoplasty: vision, astigmatism, and endothelial survival. *Ophthalmology.* 2009;116:248–256.
11. Bahar I, Kaiserman I, Levinger E, et al. Retrospective contralateral study comparing Descemet stripping automated endothelial keratoplasty with penetrating keratoplasty. *Cornea.* 2009;28:485–488.
12. Cheng YY, Pels E, Nuijts RM. Femtosecond-laser-assisted Descemet's stripping endothelial keratoplasty. *J Cataract Refract Surg.* 2007;33: 152–155.
13. Buratto L, Bohm E. The use of the femtosecond laser in penetrating keratoplasty. *Am J Ophthalmol.* 2007;143:737–742.
14. Slade SG. Applications for the femtosecond laser in corneal surgery. *Curr Opin Ophthalmol.* 2007;18:338–341.
15. Farid M, Kim M, Steinert RF. Results of penetrating keratoplasty performed with a femtosecond laser zigzag incision initial report. *Ophthalmology.* 2007;114:2208–2212.
16. Por YM, Cheng JY, Parthasarathy A, et al. Outcomes of femtosecond laser-assisted penetrating keratoplasty. *Am J Ophthalmol.* 2008;145: 772–774.
17. Price FW Jr, Price MO, Grandin JC, et al. Deep anterior lamellar keratoplasty with femtosecond-laser zigzag incisions. *J Cataract Refract Surg.* 2009;35:804–808.
18. Farid M, Steinert RF. Deep anterior lamellar keratoplasty performed with the femtosecond laser zigzag incision for the treatment of stromal corneal pathology and ectatic disease. *J Cataract Refract Surg.* 2009;35:809–813.
19. Terry MA, Ousley PJ. Replacing the endothelium without corneal surface incisions or sutures: the first United States clinical series using the deep lamellar endothelial keratoplasty procedure. *Ophthalmology.* 2003;110: 755–764; discussion 64.
20. McLaren JW, Patel SV, Bourne WM, et al. Corneal wavefront errors 24 months after deep lamellar endothelial keratoplasty and penetrating keratoplasty. *Am J Ophthalmol.* 2009;147:959–965.
21. Hindman HB, McCally RL, Myrowitz E, et al. Evaluation of deep lamellar endothelial keratoplasty surgery using scatterometry and wavefront analyses. *Ophthalmology.* 2007;114:2006–2012.
22. Dubbelman M, Sicam VA, Van der Heijde GL. The shape of the anterior and posterior surface of the aging human cornea. *Vision Res.* 2006;46: 993–1001.
23. Sicam VA, Dubbelman M, van der Heijde RG. Spherical aberration of the anterior and posterior surfaces of the human cornea. *J Opt Soc Am A Opt Image Sci Vis.* 2006;23:544–549.
24. Dubbelman M, Sicam VA, van der Heijde RG. The contribution of the posterior surface to the coma aberration of the human cornea. *J Vis.* 2007; 7:10 1-8.
25. Oshika T, Tomidokoro A, Tsuji H. Regular and irregular refractive powers of the front and back surfaces of the cornea. *Exp Eye Res.* 1998;67:443–447.
26. Rao SK, Padmanabhan P. Posterior keratoconus. An expanded classification scheme based on corneal topography. *Ophthalmology.* 1998;105: 1206–1212.
27. Shimmura S, Yang HY, Bissen-Miyajima H, et al. Posterior corneal protrusion after PRK. *Cornea.* 1997;16:686–688.
28. O'Donnell C, Maldonado-Codina C. Agreement and repeatability of central thickness measurement in normal corneas using ultrasound pachymetry and the OCULUS Pentacam. *Cornea.* 2005;24:920–924.
29. Al-Mezaine HS, Al-Amro SA, Kangave D, et al. Comparison between central corneal thickness measurements by oculus pentacam and ultrasonic pachymetry. *Int Ophthalmol.* 2008;28: 333–338.
30. Ho JD, Tsai CY, Tsai RJ, et al. Validity of the keratometric index: evaluation by the Pentacam rotating Scheimpflug camera. *J Cataract Refract Surg.* 2008;34:137–145.
31. Smolek MK, Klyce SD. Goodness-of-prediction of Zernike polynomial fitting to corneal surfaces. *J Cataract Refract Surg.* 2005;31: 2350–2355.

再生医学シリーズ[44]

角膜再生の現状と課題

羽藤　晋，坪田一男

Organ Biology Vol. 17 No. 1 2010別刷
日本臓器保存生物医学会

角膜再生の現状と課題

羽藤　晋，坪田　一男

慶應義塾大学眼科学教室

Present Status and Challenges for Corneal Regeneration

Shin Hatou, Kazuo Tsubota

Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan

The cornea consists of three layers ; the epithelium, stroma, and endothelium. Among them, stem cell research of epithelium has developed most. Functional bioengineered corneal epithelial sheet grafts from limbal stem cells has already been applied to clinical use. However, some problems such as the use of bovine serum in culture media or stem cell quality and quantity of the cell sheet, are yet to be solved. Stromal and endothelial stem cell research has not developed much compared to the epithelium. Cornea-derived progenitors (COPs) were derived from corneal stromal cells and have common characters with neural crest stem cells. Although there is much to solve, COPs may be helpful to obtain functional bioengineered stromal or endothelial graft in the future. On the other hand, new operation techniques such as deep lamellar keratoplasty or Descemet's membrane stripping endothelial keratoplasty have developed recently. As these techniques are ready to be applied to regenerative medicine, tissue engineering of the cornea are expected to catch up with preceding clinical operation techniques.

【キーワード】
角膜輪部移植，培養角膜上皮シート移植，Cornea-derived progenitors（COPs），Descemet's membrane stripping endothelial keratoplasty（DSEK）

はじめに

我が国での角膜移植における現状は，移植医療の対象となる他の臓器と共通して，深刻なドナー不足が問題となっている。厚生労働省の平成18年の調査によると，我が国において，角膜疾患による視覚障害で身体障害者認定を受けている人数は19,000人いるとされる。これらの患者さんひとりひとりは視力さえ回復すれば十分社会復帰可能と考えられるのに，それがかなわずQOLが著しく低い生活を余議なくされている事も問題であるし，日本全体としての観点で考えると労働力の欠如や社会保障費の圧迫による国家的損失は計り知

別刷請求先：羽藤　晋　〒160-8582　東京都新宿区信濃町35　慶應義塾大学医学部眼科学教室
TEL：03-3353-1211，FAX：03-3359-8302
E-mail：hatoushin@kankakuki.go.jp

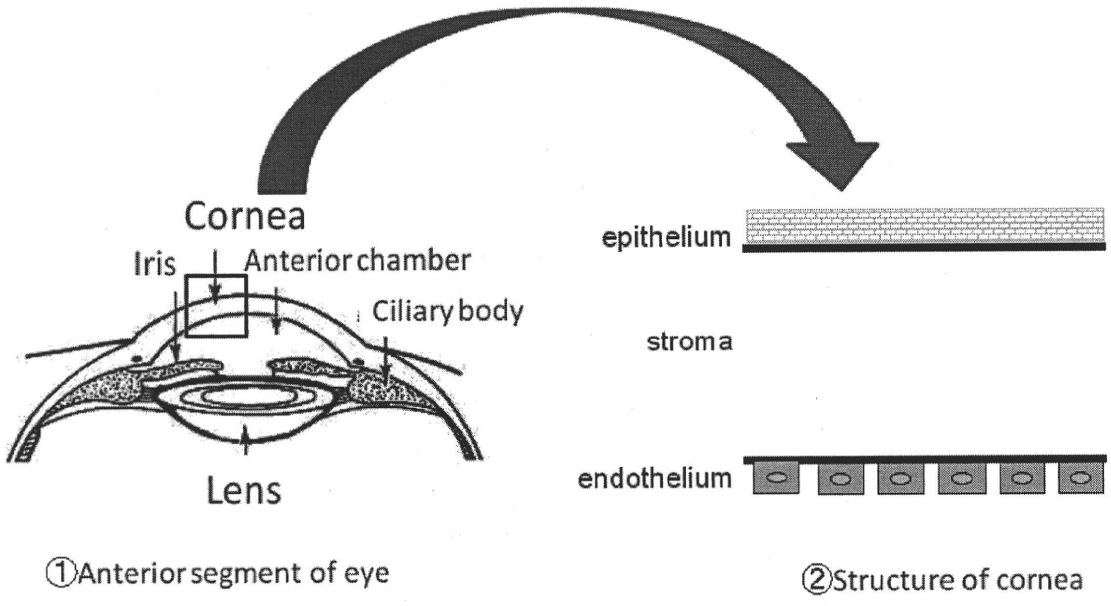

Fig. 1：Anatomical structure of anterior segment of eye and cornea.

れない。日常生活を営むには一般的に0.1以上の矯正視力が必要とされており，視力を改善し社会復帰するには角膜の全層（角膜上皮，実質，内皮）が透明に回復することが不可欠である。一方で，我が国の年間の角膜移植手術件数は1,600人前後であり，身体障害者数の10分の1に満たない。我が国の視覚障害に人口が我が国の約2倍である米国では年間約4,5000件の角膜移植が行われているが，人口の差を加味しても我が国のドナー不足の深刻な状況は歴然として存在している。

こうした我が国の角膜移植の現状に対応するには，限られたドナー角膜が多くの患者に有効利用されるような治療法の確立が急務であり，そのためには再生医学の進歩が必須であることはいうまでもない。細胞工学やバイオマテリアル技術を統合して，人工的に角膜を再生する技術の確立を，我々を含む多くの研究期間が目指している。

本稿では，角膜上皮，実質，内皮再生医療の現状と課題について，私見を交えて解説する。

1．角膜の特殊性

角膜は上皮，実質，および内皮の3層により構成され，それらの層はいずれも血管を必要としない特殊な構造をしている（Fig. 1）。無血管であるということは，角膜を再生させるためには肝臓や腎臓などの臓器とは根本的に異なるアプローチが必要となる。すなわち，ほとんどの組織では血管の再構築が大きな目標であることに対して，角膜の再生では血管を排除する必要がある。また，軟骨再生などで見られる線維芽細胞による組織補填は，角膜ではむしろ瘢痕形成による混濁の原因となる。このように，機能的な角膜を再生するには，過度な創傷治癒過程を制御しなければならない。

角膜上皮は角膜周辺の輪部に存在する幹細胞から供給された後，徐々に中心へ向かって進行し，最終的には表面から脱落する[1)]。一つの細胞が幹細胞から分裂して脱落するまでかかる時間は数週間と言われており，角膜移植をしてもおおよそ半年で上皮はすべてホスト由来の上皮に入れ替わる。一方で角膜内皮細胞は単層の扁平な細胞であり，ヒトでは脳神経細胞と同様に分裂しないと言われている。角膜内皮細胞には主にNa，K-ATPaseによるNa^+イオン能動輸送を介した強力なポンプ機能が備わっており，角膜実質を透明に維持するため，常に水分を実質から前房内に排出

している。角膜内皮細胞の幹細胞・前駆細胞についてはあまり多くはしられていない。角膜実質細胞も未だ解明されていないことが多い。他の組織に存在する線維芽細胞とは明らかに異なる特徴もあり，角膜上皮細胞の恒常性維持には不可欠と言われている。これは，角膜実質細胞と上皮細胞が常にサイトカインや成長因子を介してinteractionをしているためであり，とくに創傷治癒では重要な役割を担っていると考えられている[2]。

2．角膜移植

角膜の再生医療が発展するひとつのきっかけとなったのが角膜移植技術の進歩である。20世紀初頭から行われている角膜3層全てを移植する全層角膜移植の他に，最近ではさらに混濁した層のみを移植する「パーツ移植」が普及しつつある。角膜内皮を温存して，ドナーの上皮と実質のみを移植する深層層状角膜移植（Deep lamellar keratoplasty；DLKP）は，内皮に対する拒絶反応を予防することができる[3,4]。一方で，2000年代に入って，内皮細胞が傷害された症例では内皮を含む一部の角膜だけを移植する，いわゆる角膜内皮移植も実現化に達している。これは，角膜全体を移植するしかなかったかつての状況に比べると格段の進歩である。しかし，スティーブンスジョンソン症候群，眼類天疱瘡や角膜化学傷・熱傷などの重傷疾患はでは角膜幹細胞を含む輪部も広範囲に障害されるため，長年治療が不可能であった（Fig. 2）。1980年代になって，健眼の幹細胞を含む輪部組織を，幹細胞不全に陥った患眼に移植する角膜輪部移植が報告された[5]。自家移植では当然拒絶反応は認められず，熱傷や，化学傷の症例で片眼が健常な症例が良い適応となる。両眼とも幹細胞不全を来した症例では，ドナー角膜由来の輪部移植を行う術式が1990年代に入ってから徐々に普及した[6]。ドナー輪部に含まれる幹細胞が後にtransient amplifying cell（TAC）細胞に移行しつつ，レシピエント角膜を再生すると考えられている。移植した幹細胞が永続的に機能しているかは常に議論されている点であるが，我々は

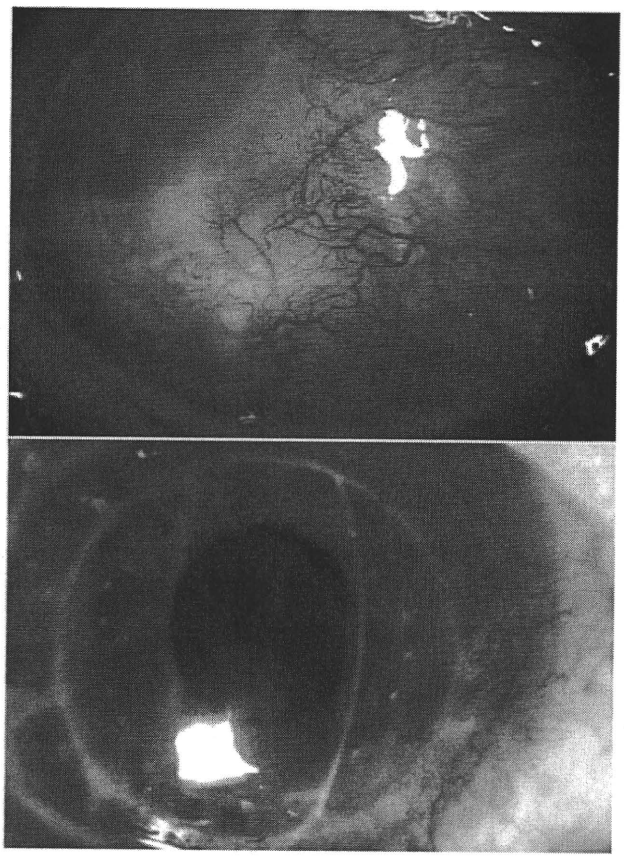

Fig. 2：The eye with ocular pemphigoid.

Superior：Stem cell deficiency in corneal limbal epithelium caused conjunctival invasion with blood vessels on whole corneal surface. Inferior：One year after the keratoplasty with limbal epithelial stem cell transplantation. Center of the cornea was covered with corneal epithelium and maintained clarity.

ドナーとレシピエントの性別が異なる症例において，一年以上経過してもドナー由来の細胞が存在することを示した[7]。

3．臨床応用された培養上皮シート移植

幹細胞不全を来した症例に対して，健常眼の角膜，あるいはドナー角膜の幹細胞を含む輪部組織を移植することが可能であることはすでに説明した。しかし，移植した幹細胞から増殖する上皮細胞による創傷治癒が遅くなると炎症が悪化し，それによってさらに上皮化が得られないという悪循環に陥る場合がある。最悪な場合は，貴重な自己組織が無駄になることもあり得る。そこで限られ

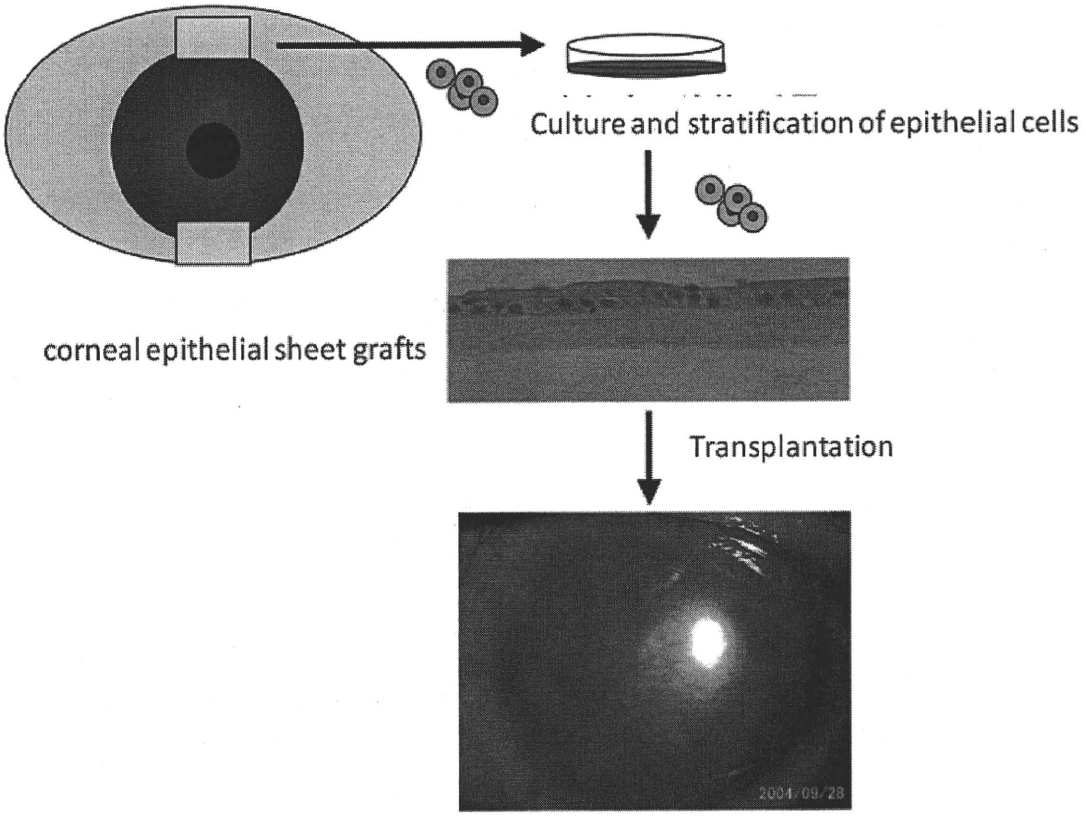

Fig. 3：The method of transplantation using functional bioengineered corneal epithelial sheet grafts from autologous corneal epithelial stem cells.

た組織で広い範囲を被覆するために，組織工学的技術によって，あらかじめ上皮をシート状に培養した再生角膜を用いる方法が開発された（Fig. 3）。これが角膜の再生医療の本格的な始まりである。

培養上皮シートを作成する技術として最初に普及したのは羊膜をキャリアーとして用いる方法で，今では最も普及している方法である[8-10]。眼表面の難治性疾患の治療に胎盤組織の羊膜を利用する手技がTsengとKimらによって報告されており[11]，それを培養上皮の代用基底膜に応用したものである。羊膜には厚い基底膜があり，Ⅳ型，Ⅴ型コラーゲンとラミニンを豊富に含むため，上皮細胞の接着・遊走に適している。角膜の遷延性上皮欠損に対して，羊膜移植を行うことによって上皮化を得られた症例を何例か経験している。これは，基底膜の環境さえ整えれば輪部機能が残っている症例で上皮化が得られることを示している。羊膜を代理基底膜として用いることで，上皮化促進効果に加え，ケラチンなどの角膜上皮固有の遺伝子が正常に分化することも報告された[12]。

羊膜は代用基底膜になるという利点はあるが，羊膜そのものは半透明であり，視機能的には十分とは言えない。そこで最近は，羊膜を用いない培養上皮シートが開発されるようになった。西田らは温度応答性ポリマーを用いてキャリアー・フリーの培養上皮シート移植をすでに臨床応用している[13,14]。同じように，フィブリンを基材として培養シートを作成し，移植時にフィブリンを融解する方法も報告されている（Fig. 4）[12]。いずれの方法も，角膜上皮基底膜が比較的温存されている症例には有効と期待されている。

培養上皮移植は少量の細胞で大きな移植片が作成できる利点がある一方で，改善しなければなら

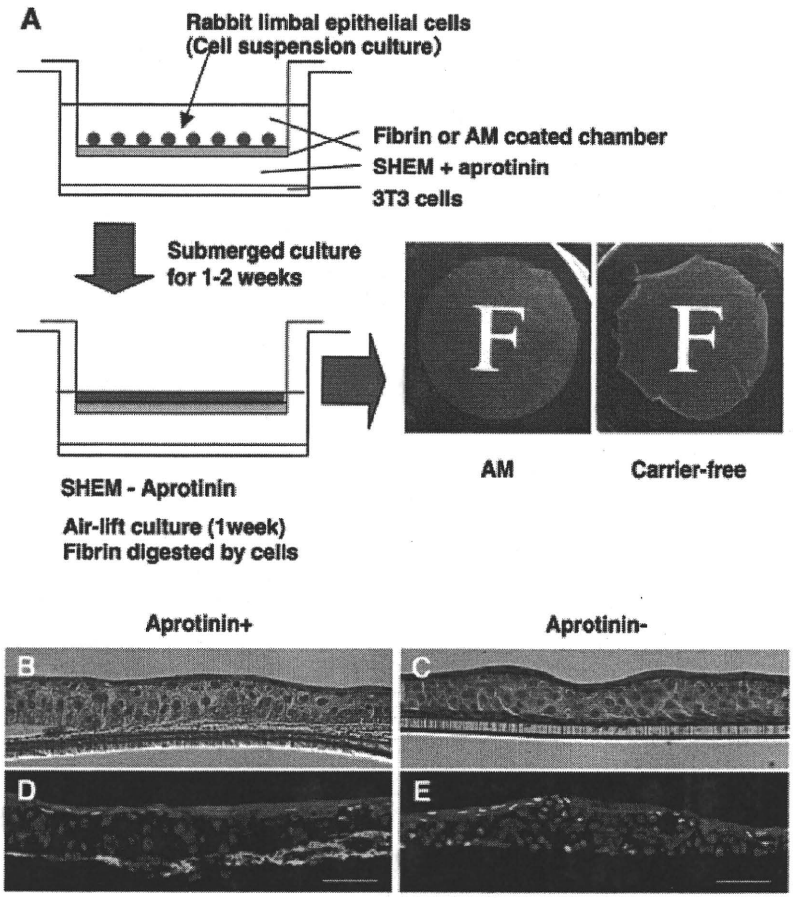

Fig. 4 : Cultivation of carrier-free epithelial sheets.

Limbal epithelial cells were collected and seeded on fibrin- or amnionic membrane (AM)-coated chambers (A). HE staining (B, C) and immunohistochemistry against fibrin (*green*) and K12 (*red*) (D, E) showed that fibrin acted as a scaffold during cultivation with the protease inhibitor aprotinin (B, D) and was allowed to dissolve by removing the aprotinin before transplantation (C, E).

ない問題もある。現在の培養方法では培養液に血清を添加する必要があるのだが，ウシ血清ではプリオンによる汚染が絶対ないという保障はなく，臨床サンプルに使用すべきでないという意見もある。将来的に無血清培地での培養上皮シート作成を目指した研究が進行中である。また，培養した上皮シート内の幹細胞の量・質の問題などまだ解明されていない点も多い。

4．角膜に存在する幹細胞

角膜再生医療の発展には，角膜に存在する幹細胞についてさらなる研究が必要である。培養上皮シートの質を担保するには，上皮幹細胞の分離・培養方法や保存方法などの進歩が必要である。また，実質と内皮も角膜の再生には不可欠であり，これらの細胞も果たして幹細胞が存在するのか，あるいは，幹細胞から分化誘導できるのかについて盛んに研究が行われている。

A）角膜上皮幹細胞

角膜上皮幹細胞の同定を試みた多くの研究は，自己複製能と長い細胞周期を示すことで幹細胞を同定する手法を用いている。Cotsarelisらは，BrdUをウサギに投与して，全ての増殖細胞核が

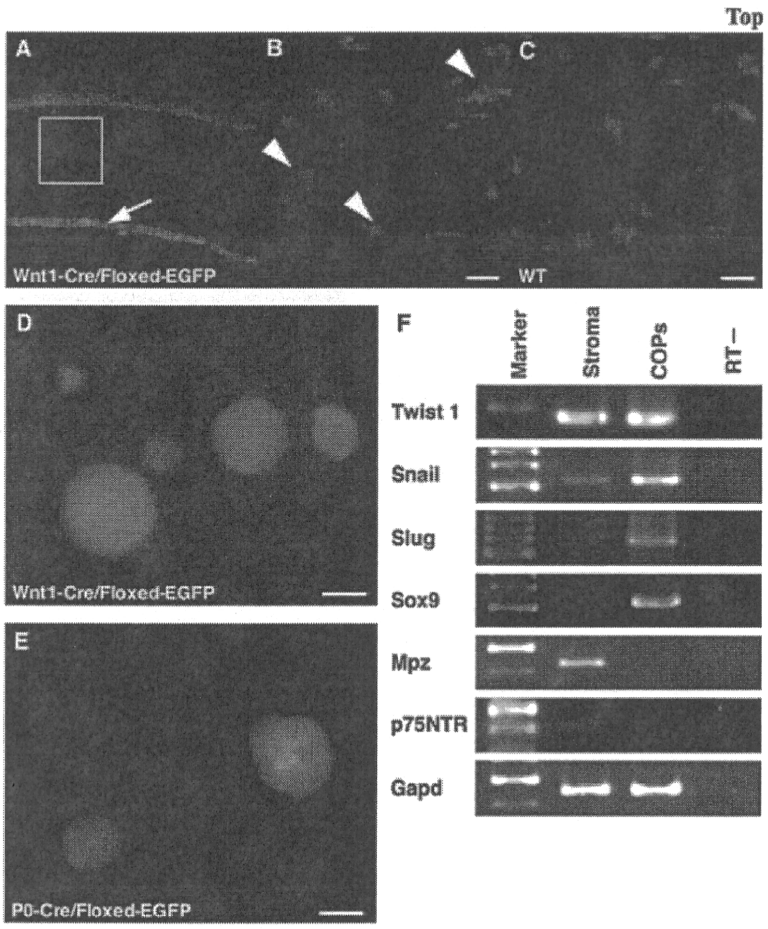

Fig. 5：Cornea-derived progenitors（COPs）.

COPs from transgenic mice encoding P0-Cre/Floxed-EGFP as well as Wnt1-Cre/Floxed-EGFP were GFP$^+$, indicating the neural crest origin of COPs, which was confirmed by the expression of the embryonic neural crest markers, *Twist*, *Snail*, *Slug*, and *Sox9*.

染色するまで持続した[15]。一定期間後（一般的には2～3ヶ月）の染色性を根拠に細胞周期が長い細胞を幹細胞であると示唆している。BrdUは細胞分裂ごとに各細胞に分散・希釈されるため，長期観測すると徐々に組織からその染色像は消失する。しかし，幹細胞のような分裂回数が少ない細胞だけBrdUによる染色が残る。この特徴から，これらの細胞をlabel retaining cell（LRC）とも呼ぶ。

一方で，自己複製能を未分化の指標とする実験系として，colony forming efficiency（CFE）が一般的に普及している。本法は，分化細胞に増殖能が残っていないことを利用して，より自己複製能が高い未分化細胞のみを培養系にて抽出する。補助役のフィーダー細胞と共培養した上皮細胞は，その増殖能力の強さによって，様々な大きさのコロニー（細胞集団）を形成する。分化細胞はコロニーを形成しないとされており，一定期間（約2週間）後に確認できるコロニーは幹細胞，あるいはその第一世代にあたるtransient amplifying cell（TAC）由来と考えられている。

幹細胞を直接識別するためのマーカー（固有な遺伝子）の探索も盛んに行われている。幹細胞マーカーと，分化した細胞のみが発現する分化マーカーを組み合わせて調べることにより，個々の細胞の分化度合いが評価できるとされている。現時

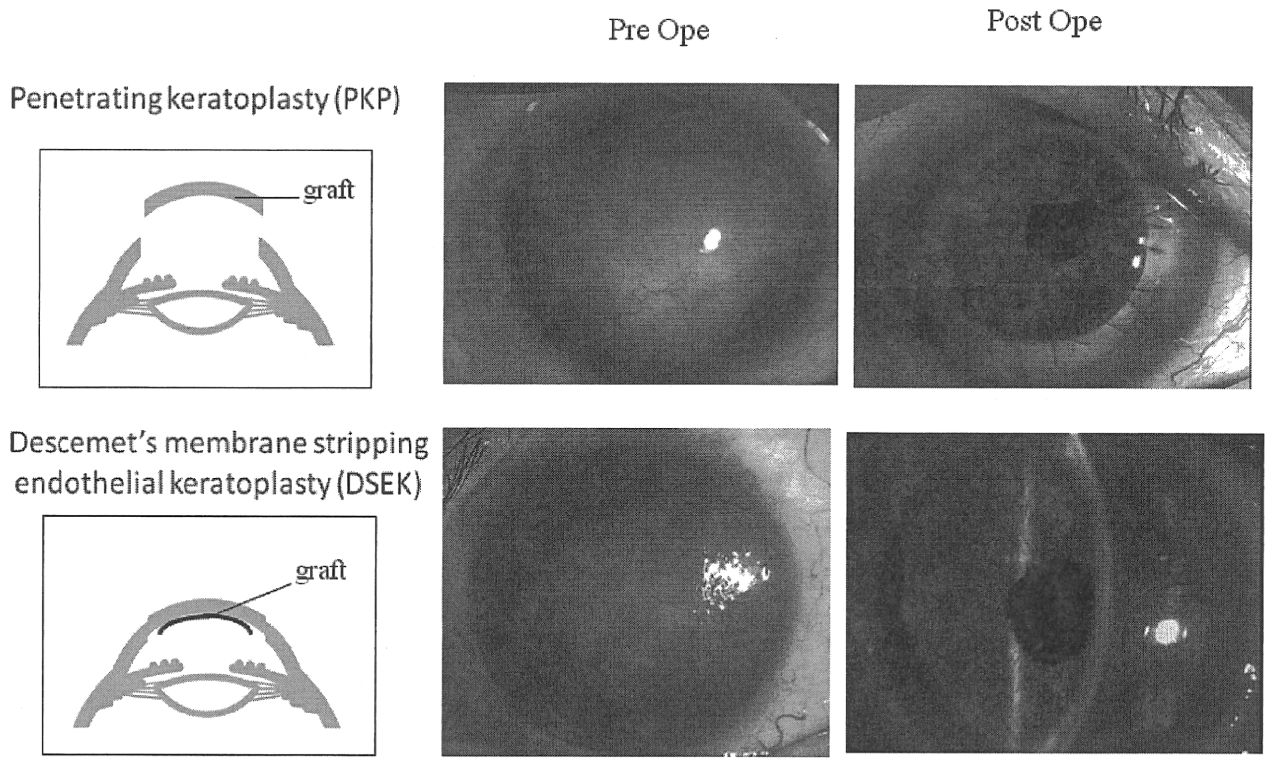

Fig. 6：Penetrating keratoplasty（PKP）and Descemet's membrane stripping endothelial keratoplasty（DSEK）.

DSEK is a new technique of sutureless corneal endothelium transplantation with small incision. This technique will be applied to regenerative medicine of corneal endothelium.

点で角膜上皮の未分化な細胞を標識するマーカーとしてp63[16]，ABCG2[17]，cytokeratin 15[18]，N-cadherin[19,20]，HES1[21]，nucleostemin[22]などが報告されている。

B）角膜実質幹細胞

角膜実質は神経提（neural crest）由来の角膜実質細胞（keratocyte）と，骨髄由来の抗原提示細胞によって構成される。実質幹細胞の研究は上皮幹細胞ほど発展しておらず，未だに報告は限られている。我々はマウス角膜実質より幹細胞の特徴をもつ角膜実質幹細胞（Cornea-derived progenitors：COPs）の分離に成功した[23,24]。本細胞は神経幹細胞を培養する際のneurosphere技術を応用することで，無血清状態で十数継代以上も維持できることを確認している。神経堤由来であるこの幹細胞は*twist*，*snail*，*slug*，*Sox9*を発現していた。また，単一細胞からも同様なsphereを形成するclonal growthを示すこと，分化誘導培地を用いることで神経細胞（βⅢtubulin+），脂肪細胞（Oil-Red O staining+）などにも分化する能力を持っていることが確認された（Fig. 5）。皮膚からも同様の神経堤由来幹細胞が分離されており，sphereを形成するなど共通の特徴を持ち備えている。しかし，これらの細胞が相互的に分化しうるのか，あるいは生体内での局在（ニッチ）などについてはまだあまり知られていない。今後はこれらの神経堤由来幹細胞のcharacterizationが進むと予想され，将来的にはCOPsを利用した，培養細胞からの角膜実質組織の構築へと発展することが期待される。

C）角膜内皮幹細胞

角膜内皮細胞も実質細胞と同様に神経堤由来で

あり，ヒトでは生後の増殖能は非常に限られている。角膜内皮細胞の極端な減少は，角膜実質と上皮の浮腫を来す水疱性角膜症となる。角膜内皮機能不全による移植適応症例は，角膜移植全体の半数強をしめている[25-27]従来は角膜内皮を含むドナーを使って，全層角膜移植（penetrating keratoplasty；PKP）を行う以外は治療する手段はなかった。しかし，最近では内皮細胞層のみを移植する角膜内皮移植術（Descemet's membrane stripping endothelial keratoplasty；DSEK）が臨床的に実現され報告されるようになった[28]（Fig. 6）。一方で角膜内皮幹細胞についての研究はまだそれほど発展しておらず，内皮移植の手術手技が先行して確立されてきている現状である。将来的に培養角膜内皮シートの作成が確立されれば，移植手技自体はすでに確立されつつあるので，その実用化は早いと考えられるが，ヒト角膜内皮細胞は*in vitro*においても増殖させることが難しく，本分野での大きな課題となっている。さらに，内皮細胞の培養や幹細胞からの誘導で得られた組織が角膜内皮として臨床応用できるためには，Na，K-ATPase，carbonic anhydraseなど角膜内皮の機能に必要な因子が発現しているだけでなく，それらの因子が角膜の含水率を十分に制御し透明性を維持できるだけの生理・生化学的機能を発揮しなければならないことも，越えなければならないハードルの一つである。角膜実質細胞（keratocyte）も角膜内皮細胞も神経堤細胞由来であることから，われわれは前述のCOPsからの内皮細胞誘導を試みており，いくつかの培養条件でNa，K-ATPase，carbonic anhydraseなどの発現を確認している。もしCOPsを利用した幹細胞研究から培養角膜内皮シート移植が臨床的に実現できれば，角膜疾患患者にとって大きなメリットとなるだけでなく，内皮機能不全が移植適応に占めるウエイトの大きさを考えると社会的意義も非常に大きいと考えられる。

おわりに

角膜の再生医療は比較的早期から臨床応用されている専門分野のひとつである。しかし，上皮以外の角膜実質，そして角膜内皮の再生医療についてはまだまだ開発の初期にある。一方で，この10年程度の間に急速に発展してきた，深部表層角膜移植，角膜内皮移植などの先行する角膜パーツ移植の手術手技は，そのまま再生医療用に応用することができると考えられ，いったん幹細胞から実質や内皮への誘導方法が確立されればそこからの臨床応用は早いのではないかと期待される。また，最近報告された多能性幹細胞（iPS細胞）[29]から再生医療用に角膜の幹細胞を誘導する研究も行っており，その成果が期待される。前述のCOPsは神経堤細胞の性格を有しているので，iPS細胞から神経堤細胞，COPsから角膜実質・内皮細胞への誘導の道筋が解明されれば，将来的にiPS細胞から角膜全層の構築が可能になるかもしれない。角膜は体内において数十年以上透明性を維持する必要があり，そのためには細胞を支持するマテリアルの開発も必要である。構造的に単純に見える角膜でありながら，光学的レンズとしては非常に複雑，かつ合理的な形状をしている。前述したとおり，本邦においては角膜移植医療における慢性的なドナー不足が問題となっており，多くの患者の社会復帰を阻んでいる。角膜の全層の再生治療が確立できれば，本邦におけるドナー不足の問題を根本的に解決できるが，それにはまだ越えなければならないハードルが存在している。道のりはまだ長いが，研究は着実に進んでいるので今後の展開に期待したい。

文献

1) Thoft RA, Friend J. The X, Y, Z hypothesis of corneal epithelial maintenance. *Invest Ophthalmol Vis Sci* 24：1442-1443, 1983
2) Wilson SE, Mohan RR, Ambrosio R, Jr., Hong J, Lee J. The corneal wound healing response：cytokine-mediated interaction of the epithelium, stroma, and inflammatory cells. *Prog Retin Eye Res* 20：625-637, 2001
3) Aggarwal RK. Deep lamellar keratoplasty-an

alternative to penetrating keratoplasty. *Br J Ophthalmol* 81 : 178-179, 1997

4) Shimmura S, Shimazaki J, Omoto M, Teruya A, Ishioka M, Tsubota K. Deep lamellar keratoplasty (DLKP) using viscoadaptive viscoelastics in keratoconus patients. *Cornea* 24 (2) : 178-181, 2004

5) Kenyon KR, Tseng SC. Limbal autograft transplantation for ocular surface disorders. *Ophthalmology* 96 : 709-722 ; discussion 722-703, 1989

6) Tsubota K, Satake Y, Kaido M, et al. Treatment of severe ocular-surface disorders with corneal epithelial stem-cell transplantation. *N Engl J Med.* 340 (22) : 1697-703, 1999

7) Shimazaki J, Kaido M, Shinozaki N, et al. Evidence of long-term survival of donor-derived cells after limbal allograft transplantation. *Invest Ophthalmol Vis Sci* 40 : 1664-1668, 1999

8) Pellegrini G, Traverso CE, Franzi AT, Zingirian M, Cancedda R, De Luca M. Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium. *Lancet* 349 : 990-993, 1997

9) Koizumi N, Inatomi T, Suzuki T, Sotozono C, Kinoshita S. Cultivated corneal epithelial transplantation for ocular surface reconstruction in acute phase of Stevens-Johnson syndrome. *Arch Ophthalmol* 119 : 298-300, 2001

10) Shimazaki J, Aiba M, Goto E, Kato N, Shimmura S, Tsubota K. Transplantation of human limbal epithelium cultivated on amniotic membrane for the treatment of severe ocular surface disorders. *Ophthalmology* 109 : 1285-1290, 2002

11) Kim JC, Tseng SC. Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. *Cornea* 14 : 473-484, 1995

12) Higa K, Shimmura S, Kato N, et al. Proliferation and Differentiation of Transplantable Rabbit Epithelial Sheets Engineered with or without an Amniotic Membrane Carrier. *Invest Ophthalmol Vis Sci* 48 : 597-604, 2007

13) Nishida K, Yamato M, Hayashida Y, et al. Functional bioengineered corneal epithelial sheet grafts from corneal stem cells expanded ex vivo on a temperature-responsive cell culture surface. *Transplantation* 77 : 379-385, 2004

14) Nishida K, Yamato M, Hayashida Y, et al. Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. *N Engl J Med* 351 : 1187-1196, 2004

15) Cotsarelis G, Cheng S-Z, Dong G, Sun T-T, Lavker RM. Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate : Implications on epithelial stem cells. *Cell* 57 : 201-209, 1989

16) Pellegrini G, Dellambra E, Golisano O, et al. p63 identifies keratinocyte stem cells. *Proc Natl Acad Sci U S A* 98 : 3156-3161, 2001

17) Watanabe K, Nishida K, Yamato M, et al. Human limbal epithelium contains side population cells expressing the ATP-binding cassette transporter ABCG2. *FEBS Lett* 565 : 6-10, 2004

18) Yoshida S, Shimmura S, Kawakita T, et al. Cytokeratin 15 can be used to identify the limbal phenotype in normal and diseased ocular surfaces. *Invest Ophthalmol Vis Sci* 47 : 4780-4786, 2006

19) Hayashi R, Yamato M, Sugiyama H, et al. N-Cadherin is expressed by putative stem/progenitor cells and melanocytes in the human limbal epithelial stem cell niche. *Stem cells (Dayton, Ohio)* 25 : 289-296, 2007

20) Higa K, Shimmura S, Miyashita H, et al. N-cadherin is required to maintain human corneal limbal epithelial progenitor cells *in vitro*. *Invest Ophthalmol Vis Sci* 50 (10) : 4640-4650, 2009
21) Nakamura T, Ohtsuka T, Sekiyama E, et al. Hes 1 regulates corneal development and the function of corneal epithelial stem/progenitor cells. *Stem cells (Dayton, Ohio)* 26 : 1265-1274, 2008
22) Kawashima M, Kawakita T, Yoshida S, Shimmura S, Tsubota K. Nucleostemin as a possible progenitor marker of corneal epithelial cells. *Mol Vis* 15 : 1162-1168, 2009
23) Yoshida S, Shimmura S, Shimazaki J, Shinozaki N, Tsubota K. Serum-free spheroid culture of mouse corneal keratocytes. *Invest Ophthalmol Vis Sci* 46 : 1653-1658, 2005
24) Yoshida S, Shimmura S, Nagoshi N, et al. Isolation of multipotent neural crest-derived stem cells from the adult mouse cornea. *Stem Cells*. 24 (12) : 2714-22, 2006
25) Kang PC, Klintworth GK, Kim T, et al. Trends in the indications for penetrating keratoplasty, 1980-2001. *Cornea*. 24 : 801-803, 2005
26) Al-Yousuf N, Mavrikakis E, Daya SM. Penetrating keratoplasty : indications over 10 year period. *Br J Ophthalmol*. 88 : 998-1001, 2004
27) Dobbins KR, Price FW Jr, Whitson WE. Trends in the indications for penetrating keratoplasty in the Midwestern United States. *Cornea*. 19 : 813-816, 2000
28) Price FW Jr, Price MO. Descemet's stripping with endothelial keratoplasty in 50 eyes : a refractive neutral corneal transplant. *J Refract Surg*. 21 (4) : 339-345, 2005
29) Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131 : 861-872, 2007

GANKA. OPHTHALMOLOGY Vol. 52 No. 10 2010

眼　科

眼科画像診断―最近の進歩―

Ⅰ．総論 -1．前眼部

9）スペキュラーマイクロスコピー

羽藤　晋　川北哲也

金原出版株式会社

9 スペキュラーマイクロスコピー

はじめに

スペキュラーマイクロスコピーとは，鏡面反射の原理を用いて，主に角膜内皮を観察する顕微鏡検査である。主に，というのは，実は鏡面反射の原理を用いれば，角膜内皮面だけでなく，角膜上皮や実質の観察も可能なのであるが，臨床面で最も検査としての意義が高いのは角膜内皮面の観察であり，現在臨床の現場で使われているスペキュラーマイクロスコープも角膜内皮面の観察用にデザインされている（**図 1**）。1918 年に Vogt がこの原理を用いて初めて角膜内皮面の観察を行い，1968 年になって Maurice が最初のスペキュラーマイクロスコープの試作機を発表したとされる[1]。その後，Laing，Bourne，Kaufman らによって改良がなされ[1]，今日のように日常診療で角膜内皮の写真が撮影できるようになり，広く普及している。

鏡面反射で観察する際，スリット光の幅が広いと実質や上皮からの散乱光が強くなってしまい，角膜内皮のコントラストが低下して観察しにくくなってしまうため，初期のスペキュラーマイクロスコピーでは，狭いスリット光を用いて撮影されていた。この方式では内皮面の撮影範囲がどうしても狭くなってしまい，初期のころはスペキュラーマイクロスコピーによる解析はせいぜい角膜内皮細胞密度くらいに限られていた[2]。最近は光の干渉を抑え解像度を上げる技術の進歩とともにスリット光の幅も広がり，より広範囲の角膜内皮面の観察が可能となったため，解析できるパラメータも増えている。

Ⅰ．スペキュラーマイクロスコピーでみる角膜内皮所見

スペキュラーマイクロスコピーでの正常角膜内皮所見を**図 2A，B** に示す。よくスペキュラーマイクロスコピーの写真で片側に黒いバンドが現れ

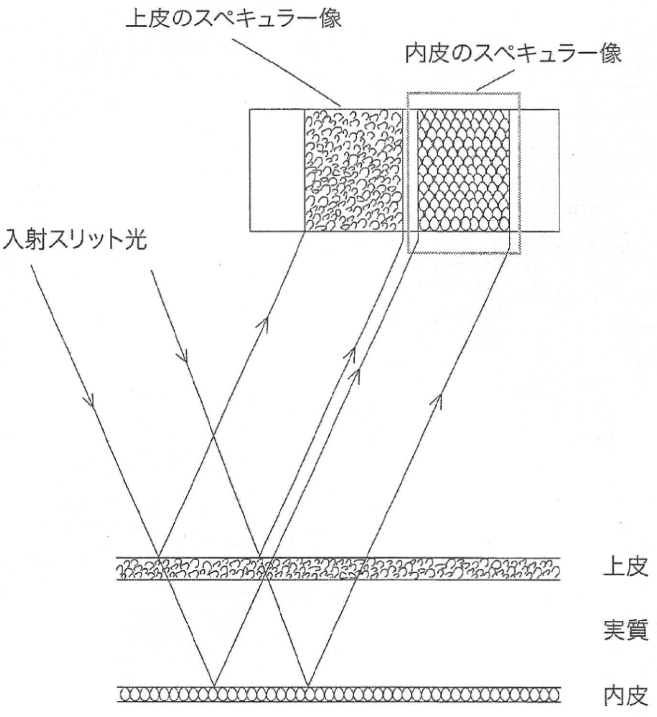

図 1
スペキュラーマイクロスコピー撮影原理の概念図。臨床で用いられる機器では角膜内皮像（□枠）のみを拾ってくるようにデザインされている。

羽藤　晋，川北哲也
Shin HATOU，Tetsuya KAWAKITA，慶應義塾大学医学部眼科学教室

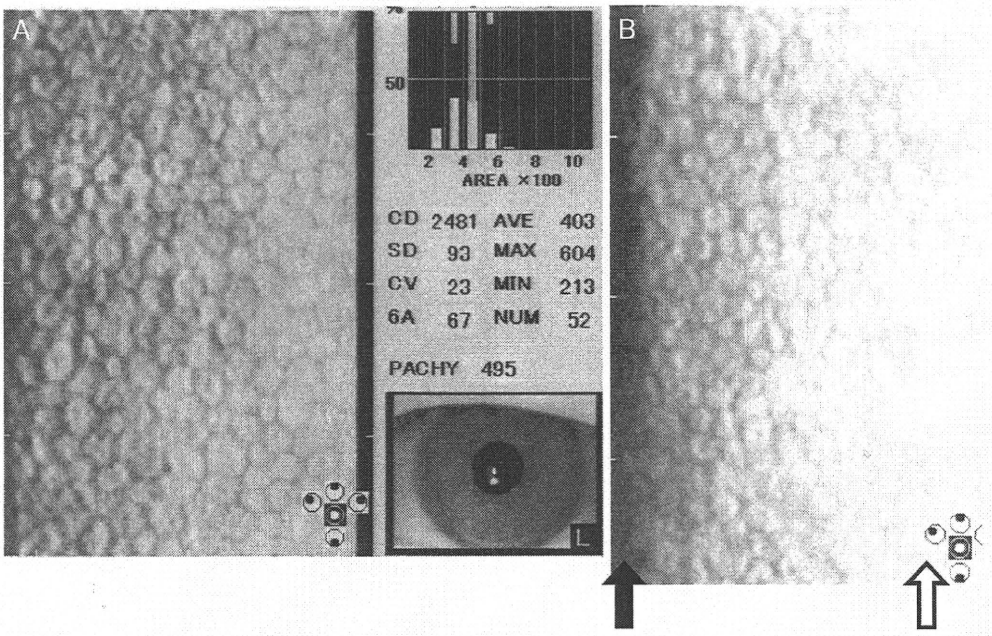

図 2
A：正常角膜内皮スペキュラーマイクロスコピー像所見。B：このスペキュラーマイクロスコピー像も正常であるが，撮影条件によっては片側に暗いバンドが出現し(黒矢印)，もう片側が輝度が高くなる(白矢印)。

たり，その反対側の輝度が高かったりするが，黒いバンドは角膜内皮と前房水との境界面によるものであり，輝度の高い部分は実質と角膜内皮の境界面の散乱光によるもので，前節で説明したスリット光での鏡面反射という観察方法上，(特に狭いスリット光で)起こりやすい現象である(**図 2B**)[1]。

スペキュラーマイクロスコピーでの角膜内皮所見では，さまざまな形状の黒い部分がみられることがあり，こうした構造物は生理的なものもあれば病的なものもある。**図 3A** のように内皮細胞内にごく小さく境界のはっきりした黒点がみられることがあるが，これは内皮細胞の微絨毛を示しているといわれ，生理的な所見である[1]。内皮細胞内にもう少し大きく境界のぼんやりした黒点がみられる場合もあるが，こうしたものは内皮細胞内の空胞や bleb を示しているといわれている(**図 3B**)[1]。虹彩炎の既往のある症例で細胞と細胞の間隙にサイズの小さい暗い構造物がみられる場合もあるが，これらは浸潤した白血球と考えられている(**図 3C**)[3]。こうした暗い構造物は Fuchs 角膜内皮変性症でみられる滴状角膜(guttata cornea)と区別されなければならない(**図 3D**)。

Ⅱ. スペキュラーマイクロスコピーの各種パラメータ

スペキュラーマイクロスコピーにおける各パラメータの意義を理解し，適切な評価をするうえで，角膜内皮細胞の生理的機能と特徴の理解は欠かせないので，簡単に確認しておきたい。角膜内皮細胞の重要な役割は角膜の含水率を一定に保ち透明性を維持することにあり，これはイオン能動輸送による pump 機能と細胞間接着分子からなるバリア機能に担われている。また，角膜内皮細胞はヒトでは増殖能がきわめて乏しく，角膜内皮細胞が何らかの影響で障害された場合，内皮細胞の増殖ではなく障害部周囲の内皮細胞の拡大，伸展により代償されるという特徴がある。角膜内皮細胞の密度という「量」的観点と，形態異常の割合という「質」的観点から，組織としての角膜内皮の機能を「推測」するのがスペキュラーマイクロスコピーである。ここで注意しなければならないのは，スペキュラーマイクロスコピーで得られる画像は角膜内皮全体のうちごく一部(そしてほとんどの場合角膜中央部)にすぎず，少ないサンプルから全体を推測しているということである。より